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Cholinergic Activation of Phosphoinositide Signaling Is Impaired in Alzheimer's Disease Brain

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JOPE, R. S., L. SONG AND R. E. POWERS. *Cholinergic activation of the phosphoinositide signal transduction system is impaired in Alzheimer s disease brain.* NEUROBIOL AGING **18**(1) 111–120, 1997.—The function of the phosphoinositide signal transduction system was compared in membranes from Alzheimer's disease (AD) and control postmortem brain. [^{3}H]Phosphatidylinositol hydrolysis was concentration-dependently stimulated by GTP[S] and this was 40% lower than controls in AD prefrontal cortical membranes. Carbachol induced a response greater than that of GTP[S] alone, and this response was impaired in AD by 45%. Differential analysis of the receptor-coupled and G-protein contributions to the responses indicated that the G-protein deficit in AD had a predominant influence on the lowered responses to cholinergic agonists. Similar deficits were observed in AD in the responses to five additional cholinergic agonists, including acetylcholine with three different acetylcholinesterase inhibitors. Deficits in stimulated phosphoinositide hydrolysis were regionally selective and these deficits did not correlate directly with reductions in choline acetyltransferase activity in AD tissues. These data demonstrate that in AD there is a brain region-selective, large impairment of cholinergic agonist-induced signal transduction mediated by the phosphoinositide system, which we speculate may impact on amyloid precursor protein processing. *Copyright © 1996 Elsevier Science Inc.*

Phosphoinositide hydrolysis Alzheimer's disease Cholinergic Muscarinic stimulation Phosphatidylinositol G-protein

ALZHEIMER'S disease (AD) comprises one of the most prevalent neurological disorders for which no adequate palliative is available (19). Among the more severe neuropathological alterations associated with AD is the progressive degenerative loss of functional cholinergic presynaptic activity. In contrast, the postsynaptic cholinergic muscarinic receptors are evidently spared (21). Therefore, a variety of agents has been used clinically or experimentally with the goal of potentiating or supplementing the degeneration-depleted endogenous acetylcholine to attempt to increase the stimulation of the postsynaptic muscarinic receptors. It is, therefore, critical to determine the functional characteristics of these muscarinic receptors in AD brain.

The phosphoinositide second messenger system is believed to be a major signal transduction system utilized by postsynaptic muscarinic receptors (8). This system consists of receptors coupled with heterotrimeric G-proteins (with α, β, and γ subunits) which, upon stimulation, activate the hydrolysis of inositol-containing phospholipids (phosphoinositides) by phospholipase C. Although multiple subtypes of G-proteins and phospholipase C exist in brain, Gq/11 and phospholipase C-β have been shown to be the predominate subtypes mediating cholinergic receptor-induced phosphoinositide hydrolysis in human brain (16,18).

In recent years, a number of investigators have examined the functional properties of muscarinic receptors in AD brain. Receptor binding methods first revealed that although the number of postsynaptic muscarinic receptors remained unaltered in AD, their coupling to G-proteins may be impaired as indicated by reduced modulation by GTP of ligand binding (9,22,29). With the recent development of methods capable of measuring receptor-coupled, G-protein–mediated phosphoinositide hydrolysis in postmortem human brain (16,18,27), deficits in the function of this signal transduction system in AD have been identified [(5,7,12,17); reviewed in (15)]. For example, this laboratory has reported severe deficits in the function, but not in the level, of the Gq/11 G-proteins mediating phosphoinositide hydrolysis in AD and a deficit in the response to the cholinergic agonist carbachol (12,17). The goal of the present investigation was to examine further the functional state of G-protein- and cholinergic agonist-induced phosphoinositide hydrolysis in AD brain.

METHOD

Tissue

Frozen samples of human brain regions were obtained from the University of Alabama at Birmingham Brain Resource Program

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directed by R.E.P., which had been coded to protect the identities of the subjects. Complete postmortem analyses were performed to ensure that control subjects were free of neurological disease and that all AD subjects met NINCDS criteria for AD. Patients with prolonged agonal states and specimens with anoxic changes were excluded from the study. Characteristics of the AD subjects (n = 6) include the following: 78 ± 1 year of age; 10.8 ± 1.8 h postmortem interval; 6 F/O M; cause of death: cardiac arrest [2], respiratory failure [2], renal failure [1], unknown [1]. Characteristics of the control subjects (n = 6) include the following : 73 ± 2 years of age; 11.0 ± 1.6 h postmortem interval; 2 F/4 M; cause of death: cardiac arrest [2], respiratory failure [4]. Thus, there was close matching for age and postmortem interval, some matching for cause of death, but sex was variable between AD and control subjects so it could contribute to differences between these groups. From autopsy and beyond, AD and control tissues were treated identically. Control and AD tissues were rapidly dissected at autopsy, individually dissected 5 mm thick slabs were rapidly frozen between blocks of dry ice, and specimens were maintained at −70°C until being thawed by immersion in ice-cold buffer for preparation of material for biochemical assays.

[^{3}H]Phosphatidylinositol (PI) Hydrolysis

The assay of [^{3}H]PI hydrolysis using human postmortem brain membranes was carried out as described previously (16,18,27). Tissue was homogenized in 6 vol of buffer A (20 mM Tris, pH 7.0, 1 mM EGTA) and centrifuged for 15 min at 20,000 × g at 4°C. The pellet was resuspended in 5 vol buffer A, incubated on ice for 15 min, and centrifuged as before. The pellet was resuspended in buffer A to a concentration of 2.5 mg protein/ml, frozen in aliquots, and stored at −80°C.

To measure [^{3}H]PI hydrolysis, membranes (0.1 mg protein) were incubated with 0.1 mM [^{3}H]PI (5–10 × 10^4 cpm; New England Nuclear) in buffer containing 10 mM Tris-maleate (pH 6.4), 6 mM $MgCl_2$, 8 mM LiCl, 3 mM EGTA with sufficient $CaCl_2$ to yield a free Ca^{2+} concentration of 0.3 μM, and other agents as noted. After incubation in triplicate for 60 min at 37° in a shaking water bath the reactions were stopped by addition of 1.2 ml of $CHCl_3$/CH_3OH (1:2, vol/vol). $CHCl_3$ (0.5 ml) and 0.25 M HCl (0.5 ml) were added, samples were mixed, and placed on ice for 10 min. Phases were separated by centrifugation, the aqueous phase was transferred to a vial for scintillation counting, and the picomoles of [^{3}H]PI hydrolyzed were calculated based upon the specific activity of the [^{3}H]PI. Blanks, obtained by deleting the incubation at 37°, were subtracted from all values. Data were analyzed for statistical significance using ANOVA with a post hoc Bonferroni test to compare values obtained from AD and matched control subjects. Differences with $p < 0.05$ were taken as statistically significant.

Choline Acetyltransferase

Brain regions were homogenized in 20 vol of buffer (50 mM NaCl, 10 mM EDTA, 50 mM NaH_2PO_4, pH 7.4) and the activity of choline acetyltransferase was measured as described by Fonnum (10).

RESULTS

The data in Fig. 1 shows that in postmortem control human prefrontal cortical membranes 3 μM GTP[S] stimulated [^{3}H]PI hydrolysis and that addition of 1 mM carbachol approximately doubled the response obtained with GTP[S] alone. Inclusion of atropine or pirenzepine blocked the stimulation by carbachol, lowering the response to that obtained with GTP[S] alone. Incubation with carbachol in the absence of GTP[S] did not activate [^{3}H]PI hydrolysis (data not shown). These results are consistent with the conclusion that GTP[S] stimulates G-proteins that activate phospholipase C, and that activation of muscarinic receptors by carbachol enhances this process in a G-protein–dependent manner, as documented previously (12,16,18).

Two deficits in the phosphoinositide response in AD prefrontal cortical membranes are apparent in the data shown in Fig. 1. First, stimulation of [^{3}H]PI hydrolysis by GTP[S] was 40% lower in AD samples than in matched controls. Second, the response to carbachol was impaired in AD. Stimulation with carbachol plus GTP[S] was 45% lower in AD, and the carbachol component (calculated by subtracting values obtained with GTP[S] alone) was 49% lower in AD.

To examine further the impaired response to GTP[S] in AD, prefrontal cortical membranes were incubated with 0.15 to 100 μM GTP[S]. The stimulation of [^{3}H]PI hydrolysis by GTP[S] was concentration dependent and was nearly maximal with 10 μM GTP[S] (Fig. 2). In AD membranes the stimulation of [^{3}H]PI hydrolysis was significantly lower than in control membranes with 0.6 μM and higher concentrations of GTP[S]. The maximal response to GTP[S] in AD membranes was 55% of that in controls. The EC_{50} for GTP[S] was approximately 0.5 μM and 1.0 μM in control and AD membranes, respectively, values equivalent to those reported previously for membranes from human brain (27) and rat brain (3).

The dependence of cholinergic agonist-stimulated [^{3}H]PI hydrolysis in human brain membranes on the GTP[S] concentration was measured using two agonists, carbachol, and pilocarpine, both of which previously have been reported to induce responses of similar magnitudes in membranes from rat and human brain (16, 23,26) and in M_1 receptor-transfected CHO cells (13). In controls the maximal [^{3}H]PI hydrolysis stimulated by each agonist was obtained with approximately 2 to 3 μM GTP[S] (Fig. 3). The EC_{50} for GTP[S] was approximately 0.2 μM with either carbachol or pilocarpine in control samples. A decrease in the GTP[S] EC_{50} induced by carbachol was reported previously in human and rat brain membranes (3,27). In AD membranes, the maximal responses to both carbachol and to pilocarpine were 50% lower in AD compared with control subjects, whereas the EC_{50} for GTP[S] was approximately 0.2 μM with both agonists, as in control membranes.

In order to negate the impact of the lower responses in AD membranes obtained with GTP[S] on those induced by each agonist, the data obtained with GTP[S] plus carbachol or pilocarpine was analyzed further by subtracting the values obtained with GTP[S] alone. This analysis (Fig. 4) demonstrated that the response induced by each agonist was, indeed, impaired in AD membranes in addition to the impaired stimulation by GTP[S]. Thus, the stimulation of [^{3}H]PI hydrolysis by carbachol and by pilocarpine (each incubated with GTP[S]) in AD membranes was approximately 50% of that in controls even after correcting for the lower response to GTP[S] alone in AD membranes.

The predominant influence of G-protein function on agonist-induced [^{3}H]PI hydrolysis and the impaired responses in AD membranes is illustrated by the data analysis shown in Fig. 5. This analysis shows the stimulation by each agonist with GTP[S] as a percent of the stimulation induced by GTP[S] alone. In control membranes, carbachol and pilocarpine stimulated the greatest percent increase over GTP[S] alone at the lowest GTP[S] concentration and as the GTP[S] concentration (and stimulation of [^{3}H]PI hydrolysis) increased the percent stimulation induced by each agonist decreased. Comparison of the results with AD and control membranes revealed that the two were remarkably similar, indicating that G-protein function dominates in controlling the re-

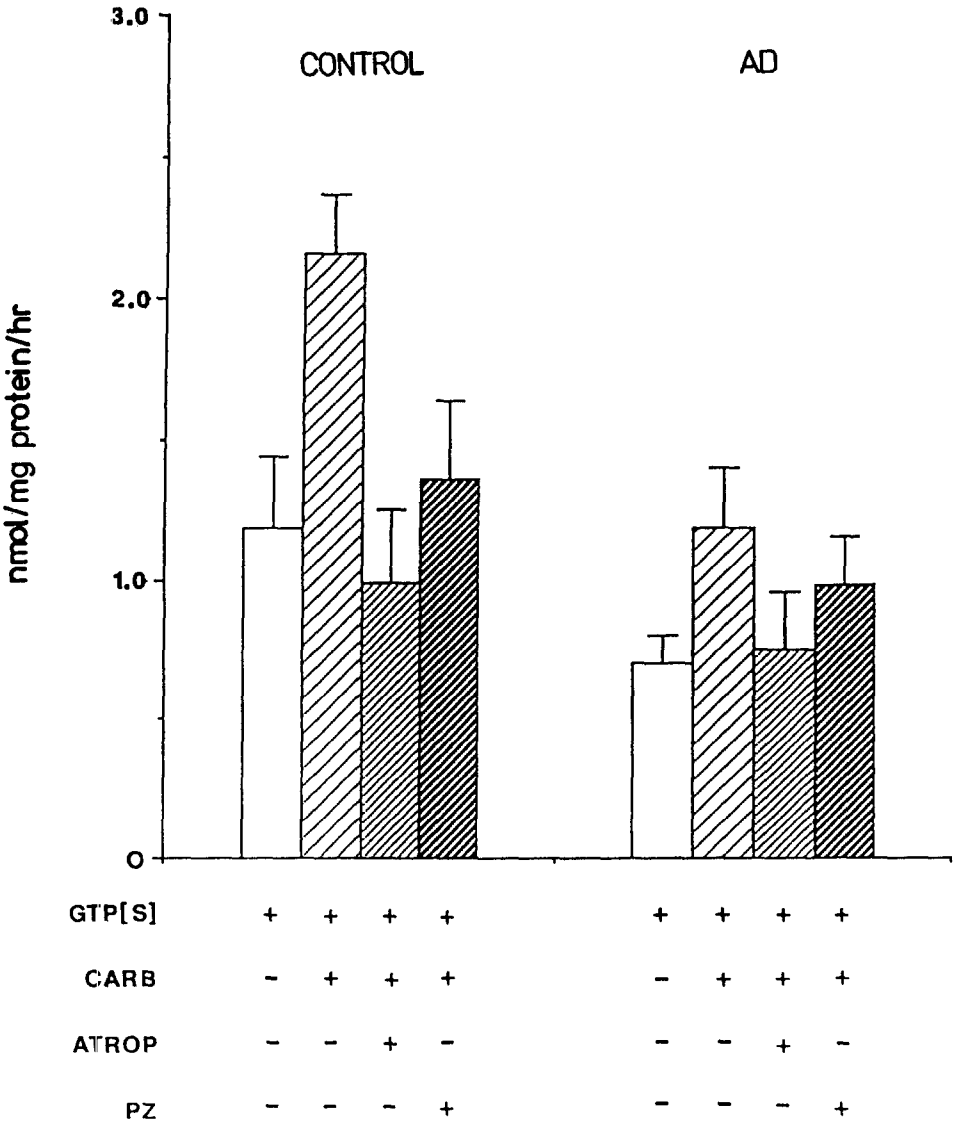

FIG. 1. [^{3}H]PI hydrolysis in membranes prepared from postmortem human prefrontal cortex. Membranes were incubated with [^{3}H]PI for 60 min at 37° as described in the Method section with 3 μM GTP[S] alone or with 1 mM carbachol (CARB) and either 1 μM atropine (ATROP) or 10 μM pirenzepine (PZ) where indicated (+). In both control and AD samples the values with GTP[S] and carbachol plus atropine or pirenzepine are not significantly different from those with GTP[S] alone. $p < 0.05$ for the responses to GTP[S] and to GTP[S] plus carbachol in AD compared with control (paired t-test). Means ± SEM ($n = 6$).

sponses to agonists, because these data are calculated as a percentage of the stimulation induced by GTP[S] alone. The largest deviation between the AD and control responses was evident at the lowest concentration of GTP[S]. With 0.15 μM GTP[S] there was little direct G-protein activation (Fig. 2), so with agonists present the majority of the [^{3}H]PI hydrolysis was dependent on the cholinergic receptor-coupled stimulation. Thus, these data demonstrate that there is a major deficit in cholinergic agonist-induced [^{3}H]PI hydrolysis in AD and that impaired G-protein activation has a prominent role in the deficient responses in AD membranes because the percent stimulation by cholinergic agonists was similar in AD and control membranes although in the former the G-protein activation by GTP[S] was 50% lower.

[^{3}H]PI hydrolysis was measured in prefrontal cortical membranes after stimulation with five different cholinergic agonists and with acetylcholine in the presence of three different inhibitors of acetylcholinesterase (Table 1). The response to bethanechol was slightly less than that of carbachol, oxotremorine-M, and pilocarpine, each of which induced similar stimulations (the apparent slightly higher stimulation with pilocarpine was the result of a small stimulation occurring in the absence of GTP[S]; data not shown). Oxotremorine induced a much greater hydrolysis of [^{3}H]PI, but this was entirely due to GTP[S]-independent stimulation and was not blocked by atropine (data not shown). The mechanism accounting for agonist stimulation of [^{3}H]PI hydrolysis in the absence of GTP[S] is unknown, but has been reported for other noncholinergic agonists (e.g., serotonin) and is apparently a nonspecific G-protein–independent reaction (16,18). Acetylcholine in the presence of acetylcholinesterase inhibitors stimulated [^{3}H]PI hydrolysis to the same extent as did carbachol, there were no

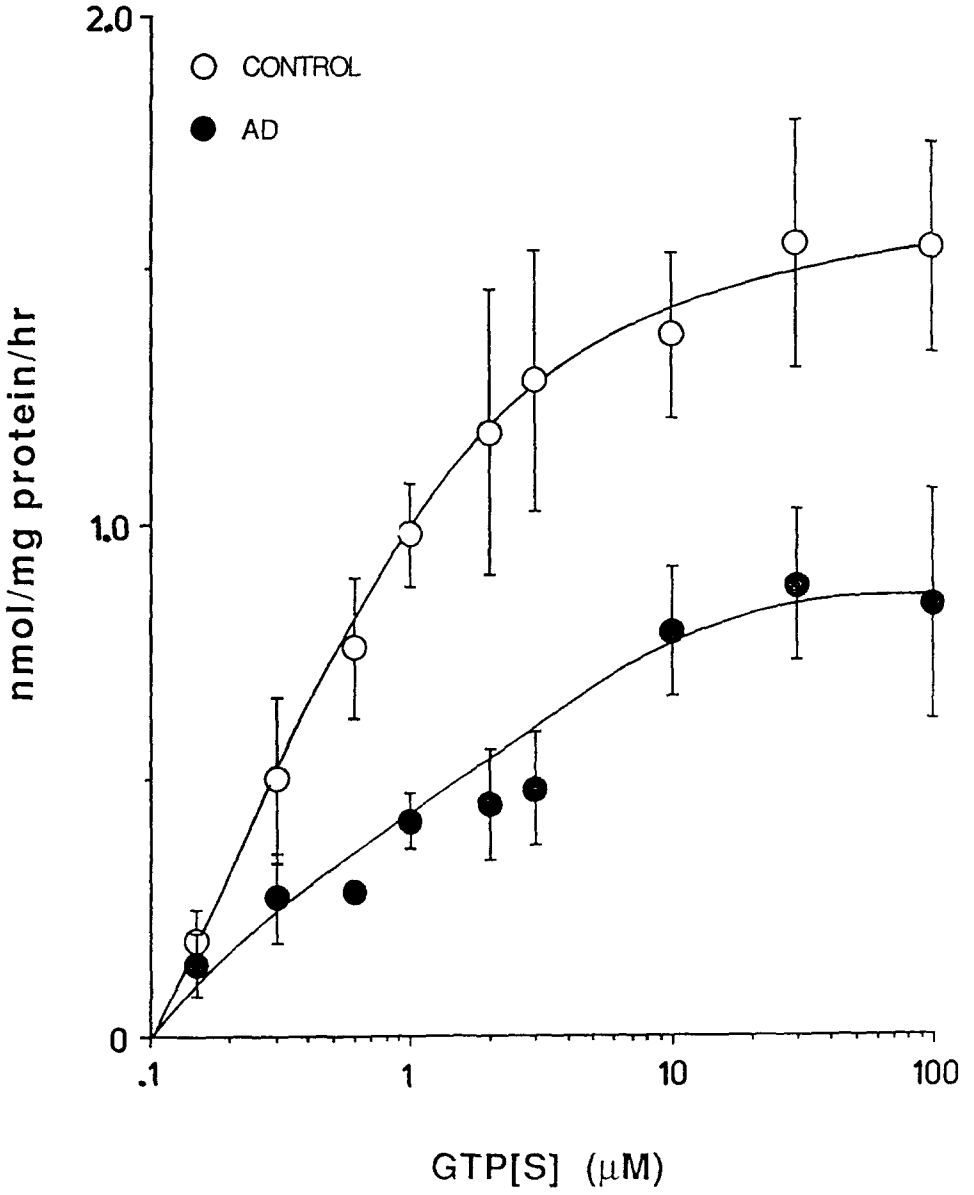

FIG. 2. GTP[S] concentration-dependent [^{3}H]PI hydrolysis in membranes prepared from postmortem control and AD prefrontal cortex. Membranes were incubated with the indicated concentration of GTP[S] and with [^{3}H]PI for 60 min at 37° as described in the Method section. Means ± SEM (n = 6). Comparison of AD to control responses revealed that $p < 0.05$ for 0.6 μM GTP[S] and all higher concentrations except $p = 0.05$ for 2 μM GTP[S] (ANOVA).

differences among the three acetylcholinesterase inhibitors that were tested, and the response was blocked by inclusion of muscarinic antagonists.

In AD membranes the relative responses to the cholinergic agonists were the same as observed in controls. However, the magnitude of cholinergic agonist-induced [^{3}H]PI hydrolysis was approximately 40 to 50% lower in AD compared with control membranes with each of the cholinergic agonists, including acetylcholine with each of the acetylcholinesterase inhibitors, with the exception of oxotremorine due to its large nonspecific effect. Thus, the deficit in the [^{3}H]PI response to cholinergic stimulation in AD prefrontal cortex was evident regardless of the agonist used.

To begin to examine the brain regional distribution of the impairment of phosphoinositide hydrolysis in AD, in addition to the prefrontal cortex, which was used in the previous experiments, assays were conducted in membranes prepared from the occipital cortex, superior temporal gyrus (STG), parahippocampal cortex, and rostral hippocampus. These measurements included testing the response to GTP[S] alone, carbachol plus GTP[S], and the glutamatergic agonist ACPD (trans-1-aminocyclopentyl-1,3-dicarboxylic acid) plus GTP[S]. ACPD was used to compare noncholinergic agonist-induced phosphoinositide hydrolysis to the responses obtained with carbachol. Stimulation of [^{3}H]PI hydrolysis by GTP[S] was approximately 50% lower in AD occipital cortex and STG compared with controls but the response to GTP[S] was similar in the two groups in the parahippocampal cortex and the rostral hippocampus (Fig. 6). The magnitude of the stimulation of [^{3}H]PI hydrolysis induced by carbachol and ACPD varied among

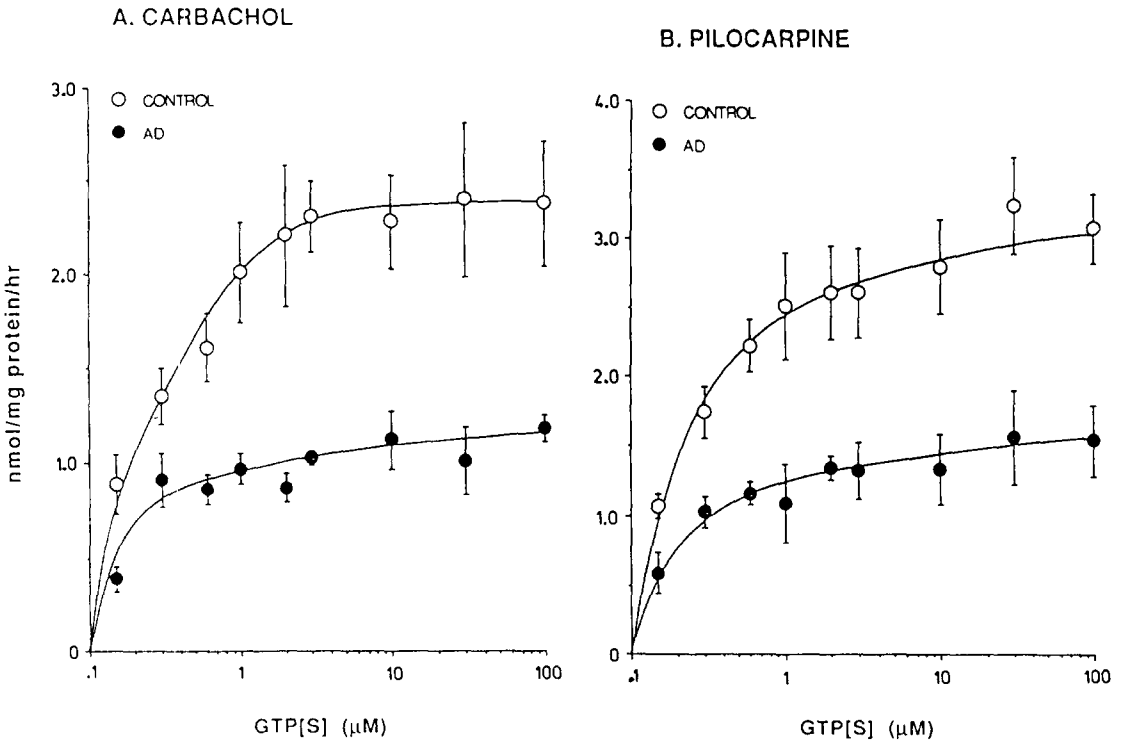

FIG. 3. GTP[S] concentration-dependence of [^{3}H]PI hydrolysis stimulated by (A) carbachol and (B) pilocarpine. [^{3}H]PI hydrolysis was measured using membranes prepared from postmortem control and AD prefrontal cortex incubated with the indicated concentration of GTP[S] and 1 mM carbachol or pilocarpine at 37° for 60 min. Means ± SEM (n = 6). Comparing AD and control responses, $p < 0.05$ for all values except $p = 0.06$ for 0.3 μM GTP[S] with carbachol (ANOVA).

TABLE 1

[^{3}H]PI HYDROLYSIS IN MEMBRANES PREPARED FROM POSTMORTEM HUMAN PREFRONTAL CORTEX

A. Cholinergic Agonists		[^{3}H]PI Hydrolyzed (nmol/mg protein/h)		
GTP[S]	Agonist	Control	AD	AD (% Control)
+	None	1.18 ± 0.17	0.55 ± 0.12	47
+	Bethanechol	1.76 ± 0.16	1.05 ± 0.14	60
+	Carbachol	2.09 ± 0.16	1.10 ± 0.18	53
+	Oxotremorine-M	2.21 ± 0.26	1.29 ± 0.22	58
+	Pilocarpine	2.87 ± 0.15	1.74 ± 0.21	61
+	Oxotremorine	8.25 ± 0.56	6.98 ± 0.50	85
−	Oxotremorine	5.89 ± 0.64	5.13 ± 0.23	87

B. Acetylcholine (ACh)				[^{3}H]PI Hydrolyzed (nmol/mg protein/h)		
GTP[S]	ACh	AChE Inhibitor	Antagonist	Control	AD	AD (% Control)
+	+	Physostigmine	—	1.86 ± 0.27	1.06 ± 0.19	57
+	+	Neostigmine	—	2.06 ± 0.31	1.07 ± 0.22	52
+	+	Tacrine	—	2.01 ± 0.27	1.23 ± 0.23	61
+	+	Tacrine	At	1.28 ± 0.23	0.60 ± 0.20	—
+	+	Tacrine	Pz	1.43 ± 0.17	0.68 ± 0.20	—
−	+	Tacrine	—	0.28 ± 0.12	0.28 ± 0.15	—

Membranes were incubated with [^{3}H]PI for 60 min at 37° and the agents indicated as described in the Methods section. The concentrations of the agents were 3 μM GTP[S], 1 mM for all agonists, 10 μM for acetylcholinesterase (AChE) inhibitors, 1 μM atropine (AT), and 10 μM pirenzepine (PZ). Means ± SEM (n = 6).

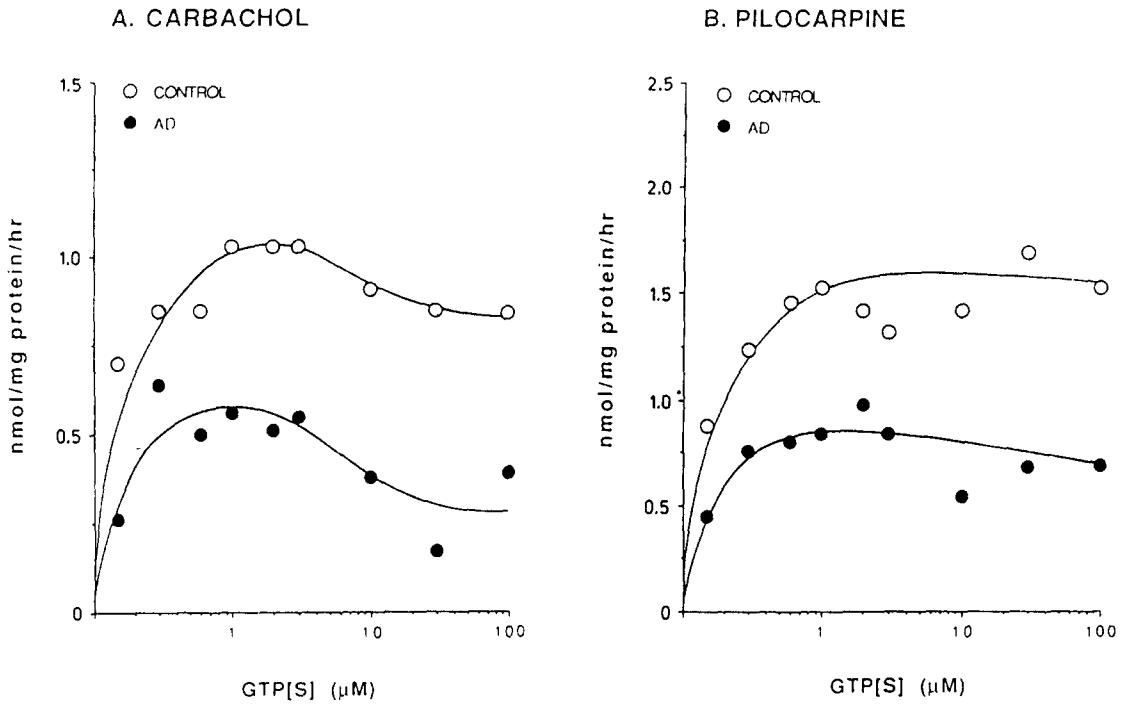

FIG. 4. Agonist-induced portion of [^{3}H]PI hydrolysis induced with GTP[S] and (A) carbachol or (B) pilocarpine in membranes prepared from control and AD prefrontal cortex. Values for [^{3}H]PI hydrolysis stimulated by GTP[S] alone reported in Fig. 2 were subtracted from those stimulated by GTP[S] plus each agonist reported in Fig. 3 to calculate the agonist-dependent component of [^{3}H]PI hydrolysis at each concentration of GTP[S]. $n = 6$.

the four brain regions, as did the influence of AD. Responses to both agonists were lower in AD occipital cortex compared with controls, whereas only the carbachol stimulation was lower in AD parahippocampal cortex, and no statistically significant effect of AD was apparent in the superior temporal gyrus and rostral hippocampus.

To examine if there was a correlation between impaired [^{3}H]PI hydrolysis and loss of cholinergic innervation, the activity of the presynaptic cholinergic marker choline acetyltransferase was measured in each brain region from Alzheimer's disease and control subjects. Table 2 shows that choline acetyltransferase was severely reduced in all brain regions that were examined from Alzheimer's disease subjects compared with controls.

DISCUSSION

The primary goal of this investigation was to examine directly in AD and control postmortem brain regions the response of the phosphoinositide signal transduction system to cholinergic stimulation. Clarification of the functional state of muscarinic receptors coupled to the phosphoinositide system in AD is important because of the limited therapeutics currently available to treat AD. Most widely applied are those drugs that inhibit acetylcholinesterase, although others that increase acetylcholine synthesis or release and that are direct agonists for muscarinic receptors are also under scrutiny. Each of these drugs is dependent upon the presence of adequate levels of functional muscarinic receptors in AD brain for there to be a therapeutic effect. Thus, direct measurements of stimulant-induced phosphoinositide hydrolysis in control and AD membranes allows for the assessment of the therapeutic potential for muscarinic agonists in AD. A direct analysis of the activity of the phosphoinositide system was not possible until recently because endogenous phosphoinositides are not adequately labeled in postmortem human brain preparations incubated with labeled precursors such as [^{3}H]inositol (11). Additionally, such assays would

TABLE 2

CHOLINE ACETYLTRANSFERASE (ChAT) ACTIVITY IN CONTROL AND ALZHEIMER'S DISEASE (AD) BRAIN REGIONS

	ChAT (pmol/min/mg protein)		
Brain Region	Control	AD	% Control
prefrontal cortex	95 ± 13	34 ± 11*	36%
occipital cortex	56 ± 3	23 ± 6*	41%
superior temporal gyrus	92 ± 16	13 ± 3*	14%
parahippocampal cortex	121 ± 27	35 ± 11*	29%
rostral hippocampus	207 ± 52	58 ± 26*	28%

$n = 5$–6; * $p < 0.05$ compared with controls.

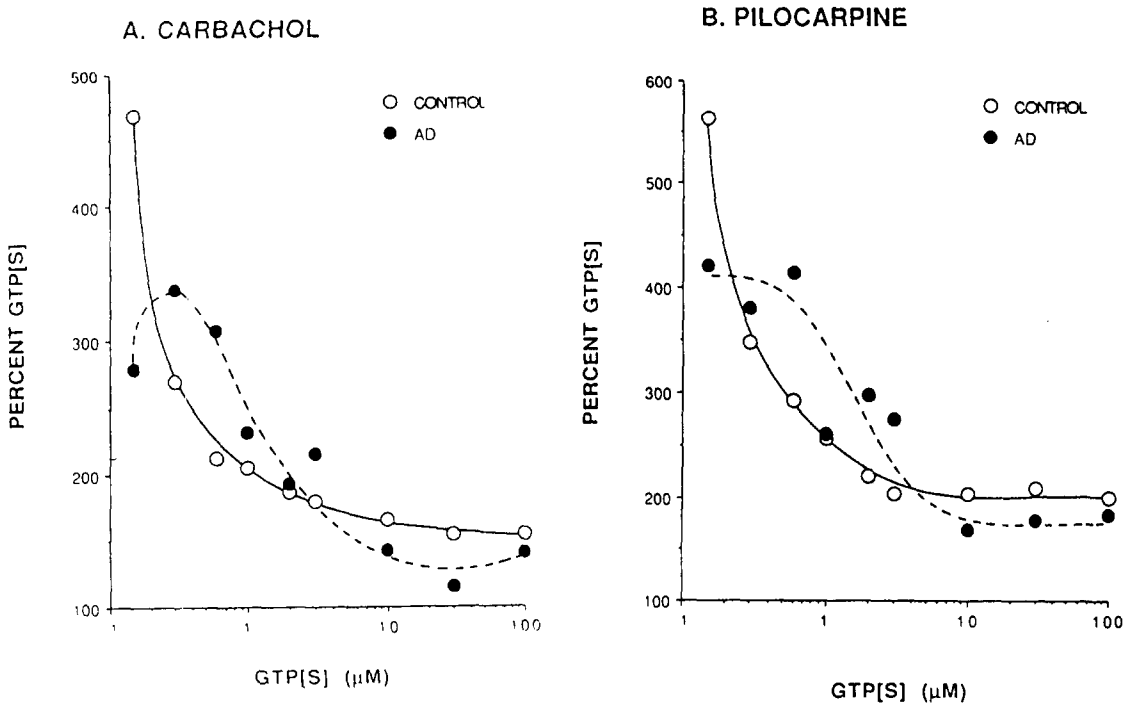

FIG. 5. [^{3}H]PI hydrolysis in control and AD prefrontal cortical membranes stimulated by GTP[S] plus (A) carbachol or (B) pilocarpine calculated as the percent of the response to GTP[S] alone. Values from Fig. 3 were divided by those in Fig. 2 to calculate agonist-induced [^{3}H]PI hydrolysis above that obtained with GTP[S] alone at each concentration of GTP[S] (n = 6).

be difficult to interpret because the concentrations of phosphoinositides are lower in AD than control brain (24). However, recent reports have demonstrated that the use of exogenously labeled phosphoinositides incubated with membranes prepared from postmortem human brain surmounts those problems (12,16,18,27). This method allows measurements of G-protein–induced and receptor agonist-induced, G-protein–mediated phosphoinositide hydrolysis in postmortem human brain membranes, and was utilized in the current investigation to compare the activity of the phosphoinositide signal transduction system in AD and control membranes. This laboratory previously reported results from a preliminary study in which GTP[S]- and carbachol-induced [^{3}H]PI hydrolysis were found to be lower in AD than in control membranes (17). That investigation was extended by examining in greater detail the dependence on GTP[S] and determining if the response varied among several cholinergic agonists and among brain regions from control and AD subjects.

In AD prefrontal cortical membranes there were clearly impaired responses to stimulation with GTP[S] and to cholinergic agonists in the presence of GTP[S] compared with controls. Thus, in agreement with studies of GTP-induced affinity shifts in binding experiments (9,22,29), there is impaired coupling of muscarinic receptors to phosphoinositide hydrolysis in AD. A primary contributory factor for this impairment can be attributed to impaired G-protein activation, which was evident when GTP[S] was used as the stimulus. GTP[S]-stimulated [^{3}H]PI hydrolysis was clearly deficient in AD prefrontal cortical membranes, producing a response that was only about 50% of that in controls. However, there is no receptor selectivity in this response, so it cannot be concluded that the G-protein impairment reflects the function of G-proteins coupled to muscarinic receptors. To attempt to examine selectively the function of G-proteins coupled with muscarinic receptors, [^{3}H]PI hydrolysis was measured with two muscarinic agonists and an extensive range of GTP[S] concentrations. The predominant influence of the impairment in G-protein function was evident when agonist responses were calculated as a percent of the stimulation induced by GTP[S] alone. In this case, the percent stimulation by carbachol or pilocarpine was equivalent in controls and AD, demonstrating that the 50% impaired G-protein activation in AD was reflected in a deficit of a similar magnitude in the response to cholinergic agonists. We previously reported that the Gq/11 subtype was the primary G-protein mediating [^{3}H]PI hydrolysis in human brain membranes stimulated by GTP[S] and by carbachol (16,18) and that the level of Gq/11 was equivalent in control and AD prefrontal cortical membranes (12,17). It should be noted that, as with Gq/11, the levels of M_1 muscarinic receptors and of phospholipase C appear not to be decreased in AD (12,21). Thus, the intermolecular interactions among these proteins, rather than their levels of expression, appear to deteriorate in AD. Taken in conjunction with the present results, these findings indicate that there is a deficit in the function, but not the level, of Gq/11 participating in phosphoinositide hydrolysis in AD. The precise nature of this G-protein impairment remains to be identified. However, it is evident that there is reduced activation even with excessively high concentrations of GTP[S] and that there is impaired coupling of Gq/11 to muscarinic receptors. One possible explanation for these deficits is that there is a loss of targeting of Gq/11 to active postsynaptic sites associated with coupling to receptors and phospholipase C. Additionally, direct measures of GTP binding to Gq/11 are required to determine if this G-protein activity is impaired in AD, which could also account for the deficits in signal transduction in AD. Such measurements have been reported for other G-proteins, and carbachol-induced GTP binding of Go and Gi was reported to be impaired in AD, although the levels of Go and Gi in AD were not different from controls (28). As with Gq/11, this deficit in other G-proteins could be a reflection of reduced coupling of receptors with G-proteins or impaired G-protein function.

The functional status of the phosphoinositide signal transduc-

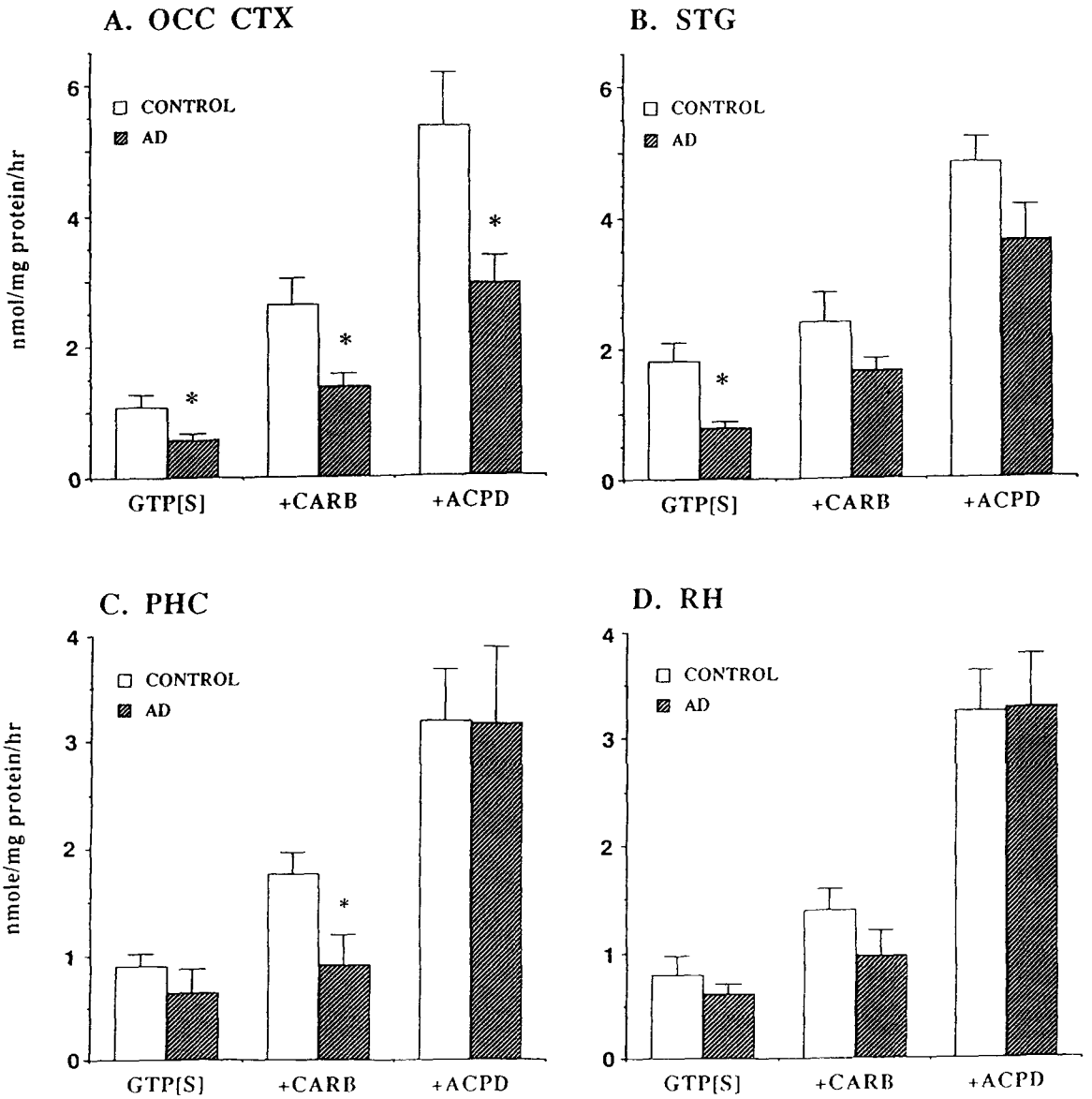

FIG. 6. Brain regional stimulation of [^{3}H]PI hydrolysis in control and AD membranes. [^{3}H]PI hydrolysis was measured using membranes prepared from the occipital cortex (OCC CTX), superior temporal gyrus (STG), parahippocampal cortex (PHC), and rostral hippocampus (RH), incubated with 3 μM GTP[S] alone or with 1 mM carbachol or ACPD. Means ± SEM (n = 5–6). *$p < 0.05$.

tion system in AD has been the subject of several reports that deal primarily with cholinergic activation but also address responses to other agonists. A deficit in cholinergic receptor-stimulated phosphoinositide hydrolysis in AD cortex, as reported here, has been observed by several investigators (5,7,12,17). The lack of finding such an impairment in AD by other investigators (1,27) may be due to different subject and tissue characteristics or different assay methodologies. These issues were recently explored in detail in a review of studies of the phosphoinositide system in AD (15). Activation of phosphoinositide hydrolysis by noncholinergic agonists, such as ACPD and serotonin (5,12), has been reported to be impaired in AD prefrontal cortex, although somewhat less than observed with cholinergic stimulation. The issue of the functional integrity of the phosphoinositide system coupled with noncholinergic receptors needs further examination. The present study revealed that the brain regional distribution of deficient ACPD-induced phosphoinositide hydrolysis appears to be more limited than the cholinergic deficit. Examination of a greater number of brain regions along with testing a wider variety of receptor agonists should provide more complete information as to how widespread are the deficits in phosphoinositide signaling in AD brain.

Although choline acetyltransferase activity was severely reduced in all brain regions examined in this study, deficits in phosphoinositide signaling did not correlate with the regional loss of choline acetyltransferase. This suggests that a mechanism other than the loss of presynaptic cholinergic input modulates the impaired phosphoinositide signaling associated with AD. We recently suggested that amyloid peptides (Aβ) may play such a role (15). Several studies have provided evidence that amyloid peptides can influence both the synthesis of phosphoinositides and their hydrolysis by phospholipase C (6,25), as well as the binding of the second messenger inositol 1,4,5-trisphosphate to its receptor (4). Additionally, phosphoinositide signaling modulates amyloid precursor protein processing such that reduced signaling activity in-

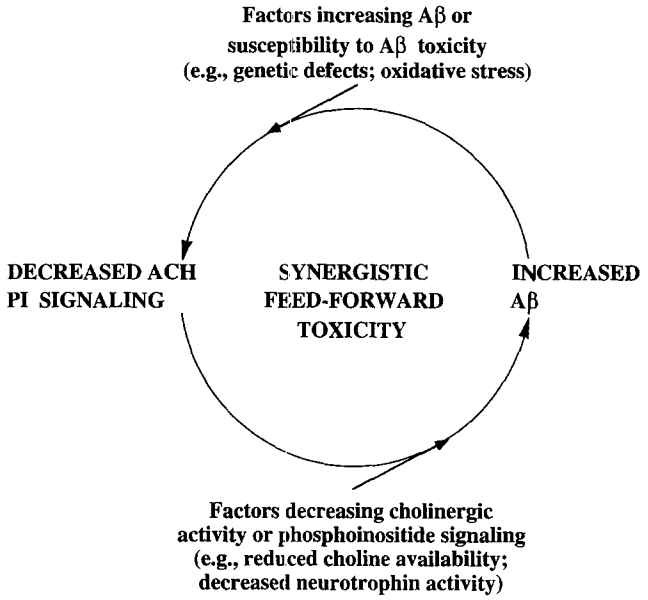

FIG. 7. Schematic representation of the interactions between the phosphoinositide signal transduction system and the production of Aβ. Factors that increase the accumulation of Aβ along with the neurotoxic effects of Aβ may contribute to decreased activity of the phosphoinositide signal transduction system in AD. Factors that decrease phosphoinositide signaling may contribute to the accumulation of Aβ. Thus, interference with phosphoinositide signaling or excessive production of Aβ may set in motion a cyclical process which exacerbates both events and contributes to progressive neuronal dysfunction.

creases Aβ [reviewed in (20)]. Because Aβ can be neurotoxic, at least partially due to oxidative stress (2,14) which we have found to inhibit phosphoinositide signaling (unpublished results), a synergistic feed-forward neurotoxic cycle may be established so that accumulation of Aβ impairs phosphoinositide signaling (through neurotoxic actions presynaptically, postsynaptically, or both), and impaired signaling exacerbates the accumulation of Aβ (Fig. 7). Thus this cyclical interaction may contribute to brain region-selective accumulation of Aβ and impaired stimulation of phosphoinositide signaling by a variety of receptor agonists.

The observation that cholinergic agonist-induced phosphoinositide hydrolysis is impaired in AD raises the question of the degree to which administration of cholinomimetics can ameliorate the cholinergic deficit. One possible solution would be to identify agents that are more efficacious than acetylcholine. Comparisons of several agonists in this study revealed little differences in their abilities to stimulate [^{3}H]PI hydrolysis in control or AD brain. However, the availability of this assay that is effective in postmortem human brains allows for the testing of newer agents that may prove to be more efficacious. A greater understanding of the regulatory mechanisms influencing G-protein function and coupling to receptors and effectors should provide insights into additional potential therapies that may enhance, or prevent deterioration of, the activity of the phosphoinositide signal transduction system in AD brain.

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